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EPA SERIES 361

SECTION HEAD



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

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JUL 15 1988

## MEMO RANDUM

Subject: Review of Comments on 2,4-D proposal not to initiate a special review

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

To: Michael McDavit, PM-62  
Special Review  
Registration Division, TS-767C

From: Marcia van Gemert, Ph.D.  
Head, Section III  
Toxicology Branch, HED, TS-769C

Management 7/15/88

Thru: Theodore M. Farber, Ph.D.  
Chief, Toxicology Branch, HED, TS-769C

Theodore M. Farber  
7/15/88

Caswell No: 315

Project No: 8-0904

Firm: Industry Task Force on 2,4-D Research Data (ITF)

The ITF submitted comments on the March 14, 1988 EPA announcement not to initiate a special review of 2,4-D. The ITF concurred with the EPA decision not to initiate a special review of 2,4-D, however, they did not agree that the chronic/oncogenicity rat and oncogenicity mouse studies should be repeated for a number of reasons. Each of the ITF arguments will be detailed below.

1. The ITF stated that the dose levels administered in the rat and mouse chronic studies were adequate to provide meaningful results. The task force argued that the doses selected for the chronic rat and mouse were based on several subchronic and pharmacokinetic studies in the rat and mouse.

EPA response: The EPA, after reviewing both the chronic/oncogenicity rat and mouse studies submitted by the ITF concluded that neither study had reached an MTD. In 1985, before the "current" EPA definition of MTD was formulated, the NTP was concerned that these studies were not being tested at a sufficiently high dose. Attached are two attachments and a letter from Jeffrey Collins, Ph.D., NTP chemical manager for 2,4-D. Attachment 1 is from a package of information which Dr. Collins presented to NTP's Toxicology Design Review Committee on Oct. 10, 1985. This document provides a description of the review by four pathologists of the relevant tissue sections from animals of the Task Forces subchronic studies, as well as the NTP's evaluation of the pharmaco-

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kinetic data utilized to set the chronic study doses.

It appears that the EPA did not "sanction" the dosage levels used in the chronic studies. No memo to the Task Force can be found stating the Branch's opinion on this matter. The letter from H. Spencer to Dr. J. Collins dated Dec. 10, 1985 actually states his opinion on the matter and the Branch policy that the responsibility of choosing dosages of test chemicals is solely the responsibility of the registrant. In addition, he stated if the study was deemed inadequate after review then the Agency would require the registrants to provide further testing. Although Dr. Spencer did not disagree with the dosage selection, he was evidently not in possession of the NTP information supplied by NTP in attachment 1 when he made his statements concerning the rationale for dosage selection.

The ITF's rationale for dosage selection appears in fact unreasonable because the kidney lesions seen in the 90-day studies upon which the chronic study doses were selected, according to the NTP pathologists, were "minimal in severity and clearly not life-threatening even at the highest dose tested in the subchronic study. No appreciable differences were observed between kidneys from control and treated female rats."

In addition, although the pharmacokinetic data, according to Dr. Collins memo in attachment 1, "did appear to support that conclusion that the active organic acid transport system involved in elimination of 2,4-D is saturated at doses of around 50 mg/kg, the kidney (glomerular) filtration mechanism does not appear to be saturated at this dose and it was not clear that saturation of the organic acid transport system resulted in any significant toxicity." Dr. Collins further noted "that the pharmacokinetic data presented was derived from gavage administration of a bolus of radioactively-labeled 2,4-D whereas the toxicologic testing of 2,4-D is being carried out by the dosed-feed route of administration."

Since there is considerable controversy between the ITF and the NTP pathologists surrounding the magnitude and severity of the kidney lesions seen in the subchronic as well as the chronic rat and mouse studies and the MTD issue hinges on this, the EPA proposes that the ITF submit the kidney slides of these controversial studies to the EPA for independent pathologic analysis.

2. The ITF stated that the NTP did not conduct a complete review of all slides.

EPA response: If in fact the NTP pathologists did not review all relevant slides on the subchronic as well as the chronic slides, this oversight will be remedied by an independent pathology review.

3. The ITF stated that the concept and definition of MTD is evolving.

EPA response: While it is true that the MTD policy has evolved to some extent over the past few years at EPA, it is felt that the present policy is scientifically sound and supportable. Concerning

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the dose selections for 2,4-D chronic studies, even NTP in 1985 felt the doses were not reasonable and that the "Task Force chronic studies are probably not being performed at an MTD." This is the same position EPA is presently taking with its "current" MTD policy.

The 2,4-D toxicology data base was evaluated this past year as part of the re-registration process for 2,4-D. During the Registration Standard process of a compound, the toxicology data base is evaluated and judged according to today's scientific standards. The acceptability of the long-term study dose selection under this Registration Standard process would be judged by the present EPA MTD policy, along with all the other chemicals up for review as Registration Standards.

4. The ITF stated that the EPA notice had stated that at interim sacrifice of 53 weeks an apparent treatment-related increased incidence of astrocytomas was observed in male animals. In fact no astrocytomas were seen at the interim sacrifice and EPA was in error.

EPA response: EPA agrees that it was in error.

5. The ITF states that 2,4-D has a relatively low order of toxicity with minimal exposure.

EPA response: While the Toxicology Branch cannot speak to the aspects of exposure, the compound does have a low order of toxicity in short-term tests. However, the issues surrounding the chronic/oncogenicity studies still need to be resolved.

6. The report entitled "report on the adequacy of high dose selection for 2,4-D oncogenicity studies" was prepared for the ITF by the consulting firm CanTox Inc. and reiterates essentially the same arguments presented by the ITF in their comments.

Conclusions: The arguments presented by the ITF concerning the magnitude and severity of the kidney lesions which were seen in both the subchronic and chronic studies were persuasive enough to warrant reconsideration by EPA of its position. The EPA requests that the ITF submit all the kidney slides from the relevant subchronic and chronic rat and mouse studies. These slides will be given to an independent contractor for pathologic evaluation.



## DEPARTMENT OF HEALTH &amp; HUMAN SERVICES

Public Health Service

October 6, 1987

National Institutes of Health  
National Institute of  
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Dr. Marcia van Gemert  
U.S. EPA  
Toxicology Branch (TS-769C)  
Office of Pesticide Programs  
401 M Street, SW  
Washington, DC 20460

Dear Marcia:

Pursuant to our recent phone conversation, I have attached the relevant portions of two documents which clearly describe the National Toxicology Program's (NTP) previous evaluation of the data (particularly the histopathologic findings) generated by the subchronic studies of 2,4-Dichlorophenoxyacetic acid (2,4-D) carried out under the sponsorship of the Industry Task Force on 2,4-D Research Data (ITFRD), data which was used to select doses for the subsequent ITFRD-sponsored chronic studies of 2,4-D.

Attachment I is from the package of information which I presented to the NTP's Toxicology Design Review Committee on October 10, 1985, and provides a relatively detailed description of the review by four NTP pathologists of the relevant tissue sections from animals of the ITFRD's subchronic studies, as well as the NTP's evaluation of the pharmacokinetic data utilized to set the chronic study doses. Attachment II, which is derived from a package of materials submitted by me to the NTP on March 2, 1987 in support of my conversion from an Expert appointment to a permanent Civil Service position, further describes the history of NTP's consideration of the testing of 2,4-D, including a summary of our evaluation of the subchronic data used to select the ITFRD chronic study doses, as well as subsequent input from the EPA.

I believe the attached materials provide the information you requested. If I can be of any further assistance in this matter, please don't hesitate to contact me. I would appreciate your letting me know as soon as the EPA makes a final decision as to how they are to proceed with additional testing of 2,4-D.

Sincerely,

A handwritten signature in cursive script that reads "Jeff Collins".

Jeffrey J. Collins, Ph.D.  
Chemical Manager, 2,4-D

Attachments

cc: Dr. E. McConnell  
Dr. J. Selkirk

## Attachment I

Studies Sponsored by the Industry Task Force on 2,4-D Research Data:

The Industry Task Force on 2,4-D Research Data was established in 1980 in response to a Call for Data by the EPA. Over the past 5 years this Task Force has sponsored various acute, subchronic, chronic and special studies on 2,4-D in rats and mice. After reviewing summaries of the results provided by the Task Force, serious concerns arose with respect to the doses selected for the current chronic dosed feed studies of 2,4-D being carried out by the Task Force in F344 rats (0, 1, 5, 15, or 45 mg/kg) and B6C3F<sub>1</sub> mice (0, 1, 15, or 45 mg/kg). These doses were selected primarily on the basis of pharmacokinetic data and on the results of the Task Force's subchronic dosed feed studies, particularly the induction of kidney pathology in rats and mice at the higher doses tested (rats [two studies] - 0, 1, 5, 15, 45, 60, 100 or 150 mg/kg; mice [one study] - 0, 5, 15, 45 or 90 mg/kg). It should be noted that other than the kidney lesions and some alterations in certain organ weights, no signs of toxicity were apparent in rats or mice in the Task Force's subchronic studies.

In light of the concerns with the Task Force's dose selection rationale for their chronic studies of 2,4-D, a meeting was held at NIEHS on August 13, 1985 at which representatives of the Task Force presented data from their subchronic and pharmacokinetic studies of 2,4-D which were instrumental in selecting doses for the current chronic studies. In addition, some results from the 1-year interim sacrifices of rats and mice in the 2-year chronic study were presented. Lastly, the Task Force made available slides of the kidney lesions observed in their subchronic studies for examination by NTP pathologists.

This exchange of information appears to have validated the original concerns of the NTP with the doses selected for the Task Force's 2,4-D chronic studies. NTP pathologists reviewed kidney sections of male and female rats from the 0, 15, 60, 100 and 150 mg/kg dose groups of the subchronic study. They were unanimous in evaluating the kidney lesions as minimal in severity and clearly not life-threatening, even at the highest dose tested in the subchronic study. No appreciable differences were observed between the kidneys from control and treated female rats. An increased incidence of epithelial regeneration was present in males from the higher dose groups. In all treated male rats there was an altered tinctorial property of the cytoplasm of the tubular cells not present in controls, but this change was not useful for separating the different dose groups. The cause of this alteration was not determined, although it may reflect either decreased cytoplasmic  $\alpha_2$ -globulin in treated males or an artifact of fixation or staining. NTP pathologists did not consider this finding to be a chemical effect on the kidney.

The pharmacokinetic data presented did appear to support the conclusion that the active organic acid transport system involved in elimination of 2,4-D is saturated at doses ~50 mg/kg. However, the kidney (glomerular) filtration mechanism does not appear to be saturated at this dose and it was not clear that saturation of the organic acid transport system resulted in any significant toxicity. It should further be noted that the pharmacokinetic data presented was derived from gavage administration of a bolus of radioactively-labeled 2,4-D whereas the toxicologic testing of

2,4-D is being carried out by the dosed-feed route of administration. Thus, the doses being examined may not be directly comparable and given the intermittent feeding by rodents and the rapid clearance of 2,4-D via the urine it seems likely that the level of 2,4-D which the test animals are actually exposed to in the Task Force's 2-year chronic studies will be well below saturation of the active elimination system. While the Task Force stated at the meeting that results of studies with the related compound 2,4,5-T were also used in selecting doses for the 2,4-D chronic studies, this was not mentioned in the formal dosage selection rationales contained in the study protocols for the chronic studies provided previously by the Task Force.

The 1-year interim sacrifice data presented indicates minimal toxicity in rats and mice at the high dose tested (45 mg/kg). Increased kidney weights were observed in high dose male rats and high dose female mice but even less kidney pathology was seen at 45 mg/kg after 52 weeks than had been evident at this dose in the previous 13-week subchronic studies. Some data was presented on terminal sacrifice rats (mice are in the second year of the study) which indicated no effects on survival. While a decrease in final body weights in high dose female rats was claimed, the data did not appear to support this conclusion.

In summary, after analyzing the data presented by the 2,4-D Task Force which was utilized in selecting doses for the current 2-year chronic studies of this chemical, including direct examination of the relevant tissue sections from the subchronic studies, the consensus of the NTP participants is that the Task Force chronic studies are probably not being performed at the MTD. Considering that three dose levels are being tested in the mouse chronic studies and four were tested in the rat chronic studies, it is inexplicable why a broader range of doses was not examined, particularly in the latter case where significant toxicity at a higher dose would still have allowed a three dose study. In light of this conclusion, the course of action for NTP testing of 2,4-D described in the following section is recommended.

#### Rationale for Testing Recommendations:

Despite the considerable toxicity testing of 2,4-D which has previously been carried out (see Background), including that sponsored by the Industry Task Force on 2,4-D Research Data, it is clear that the possible carcinogenicity of this compound still remains undefined, in accord with IARC's earlier conclusion (1,47a). Thus, with the goal of providing adequate data for assessing the carcinogenicity of 2,4-D, it is recommended that the NTP perform prechronic testing of this compound. The purpose of the 14-day repeated administration and 13-week subchronic testing will be to ensure appropriate dose selection for possible 2-year chronic studies. However, it is recommended that no decision be made on whether the NTP will carry out 2-year chronic studies of 2,4-D until the final reports of the Task Force-sponsored chronic studies are available (estimated dates: F344 rats - 6/86; B6C3F<sub>1</sub> mice - 6/87). In light of the fact that the Task Force sponsored no 14-day repeated administration studies, but instead selected doses for the subchronic studies on the basis of a variety of acute toxicity studies, it is particularly important that

repeated administration studies be included in the NTP prechronic testing so as to facilitate accurate dose selection for the subsequent 13-week subchronic studies. It is quite possible that this approach will result in higher doses being selected for the subchronic studies than were used in the Task Force-sponsored 13-week studies. It is also proposed that neurobehavioral testing be included in the subchronic studies given the reported effects of 2,4-D on the central nervous system (24-26) and on neuromotor functions (14). It should be noted that the only Task Force-sponsored neurotoxicity studies of 2,4-D were carried out in F344 rats exposed for 3 weeks by the dermal route but that no dosed feed neurotoxicity studies have been performed.

Although human exposure to 2,4-D occurs by three primary routes, dermal, oral and inhalation (with an apparent order of importance of dermal > oral > inhalation [117]), it is proposed that testing be carried out by only one route since the available data demonstrates clearly that systemic exposure to 2,4-D can be achieved by all these routes of administration. Thus, the most convenient route providing systemic exposure has been selected and it is proposed to carry out the prechronic testing of 2,4-D by the dosed feed route of administration. Note that the Task Force-sponsored subchronic and chronic studies of 2,4-D have also used the dosed feed route of exposure.

## Attachment II

## 5. 2,4-Dichlorophenoxyacetic acid (2,4-D)

Subsequent to the nomination of 2,4-D to the NTP by Dr. Rall, Director, NIEHS/NTP, I was asked by Dr. Kluwe to assume Chemical Manager responsibilities for this chemical in December, 1984. In the course of preparing a package on 2,4-D for presentation to the TDRC, I discovered the existence of an Industry Task Force on 2,4-D (denoted Industry Task Force on 2,4-D Research Data [ITFRD]), constituted in 1980 in response to a Call for Data by the EPA. I subsequently established an ongoing communication with a member of the ITFRD, Dr. David Eisenbrandt of the Dow Chemical Co. At my request, he provided me with certain materials which summarized the various acute, subchronic, chronic, and special studies of 2,4-D that the ITFRD had sponsored over the previous 5 years. Review of these materials raised concerns on my part as to the adequacy of the doses selected for the then current (mid-1985) ITFRD-sponsored chronic dosed-feed studies of 2,4-D being carried out in F344 rats and B6C3F<sub>1</sub> mice. Additional review by Dr. Montgomery, NTP, affirmed these concerns and concluded that to properly evaluate the dose setting criteria used for the chronic studies it would be necessary to examine directly the microslides of the kidney lesions from the 90-day studies which represented the major basis for the chronic doses selected.

Because of the above concerns, and after consultation with additional staff at NTP, including Drs. Bristol (then Acting Chief, CTEB) and Chhabra, I proposed to Dr. Eisenbrandt in July, 1985, that ITFRD representatives make a presentation to appropriate NTP staff in order to clarify the dose selection rationale used for their chronic studies of 2,4-D, including a presentation of data from their previous subchronic studies. In addition, data from the 1-year interim sacrifices of their current 2-year chronic studies in rats and mice could be presented. Lastly, I suggested that representative slides of the kidney lesions observed in the

ITFRD-sponsored subchronic studies, which were instrumental in selecting doses for their chronic studies, be brought to NIEHS for evaluation by NTP pathologists.

After considerable negotiations, the ITFRD accepted my proposal and a meeting was held at the NIEHS on 8/13/85. Present were six members of the ITFRD (Drs. R. Fears, D. Eisenbrandt, R. Nolan, and R. Kociba of Dow Chemical Co., Dr. D. Serrone of Biotech, and Dr. R. Wilson of PBI/Gordon Corp.) and approximately 15 NTP staff members. The ITFRD members presented results from their subchronic and pharmacokinetic studies of 2,4-D (this was the first opportunity that I or other NTP staff had had to review actual data from the ITFRD studies), discussed the rationale for dose selection for their 2-year studies, and presented some interim results (1-year) from their ongoing 2-year studies of 2,4-D in rats and mice. In addition, that same day, but prior to the meeting, Drs. Boorman, Montgomery, Elwell and Uraih, NTP, examined slides of the kidney lesions observed in the ITFRD-sponsored subchronic studies, these lesions being among the major criteria used for dose selection for the ITFRD-sponsored chronic studies.

Based on the presentations and observations at the ITFRD-NTP meeting, as well as the histopathologic review conducted by NTP pathologists, my previous concerns as to whether the ITFRD-sponsored 2-year chronic studies of 2,4-D in rats and mice were being performed at appropriate dose levels (i.e., at doses approximating the MTD) were not only supported by other NTP staff, but, if anything, heightened. This was subsequently reflected in my recommendations to the TDRC on 10/10/85, which called for the NTP to proceed with prechronic testing of 2,4-D, including neurobehavioral studies, with no decision to be made on chronic testing until results of the ITFRD-sponsored 2-year studies were evaluated. While the TDRC approved the proposed study design, I was asked to obtain further input from the EPA before a final decision could be made as to whether or not the NTP should proceed with prechronic testing of 2,4-D.

I wrote to Dr. Henry Spencer of the EPA on 10/21/85 (with copies to Drs. T. Farber, J. Moore, and L. Rosenstein of the EPA) and requested that the EPA indicate whether they approved of the study design of the ITFRD-sponsored chronic studies of 2,4-D, particularly the doses being tested, and whether the results of these studies, whether positive or negative for carcinogenicity, would be accepted as valid by the EPA. In addition, if EPA agreed that further testing of 2,4-D was warranted, I requested input as to EPA's position as to the most appropriate means of performing such studies (e.g., the NTP, the ITFRD, other?). I also provided him with a copy of my 2,4-D package presented to the TDRC on 10/10/85.

Dr. Spencer of the EPA replied in a letter (dated 12/10/85) that while the EPA had no specific objections to the design of the ITFRD-sponsored chronic studies of 2,4-D, it was their general "policy that the responsibility of choosing dosages of test chemicals was solely that of the registrant." Furthermore, the EPA proposed that no further testing of 2,4-D be conducted until the results of the ITFRD chronic studies could be reviewed and evaluated. If the study was deemed inadequate at that time, then the EPA would require the registrants to provide further testing. Based on this response from the EPA, Drs. Rall and McConnell decided on 12/17/85 that any plans for NTP studies of 2,4-D should be deferred until further notice, a decision which was reaffirmed to me by Dr. McConnell in September, 1986. It should be noted that preliminary evaluation (August, 1986) of the data from the ITFRD-sponsored 2-year studies of 2,4-D in F344 rats indicated a statistically significant increased incidence of brain tumors (astrocytomas) in high-dose (45 mg/kg) males. The EPA is currently conducting an independent histopathologic evaluation of this study.

In the meantime, I have submitted an expanded and modified version of my TDRC package on 2,4-D as a review article entitled, "The Toxicology of 2,4-Dichlorophenoxyacetic Acid (2,4-D)" (Appendix II-5) for publication in Reviews of Environmental Contamination and Toxicology. It is currently undergoing review by this journal. In addition, at the request of Dr. Canter, NTP, in October, 1986, I provided Dr. Fraumeni of the NCI with a package of material on 2,4-D, including my TDRC package, my letter to EPA of 10/21/85, EPA's response of 12/10/85, and the 8/86 summary of the ITFRD's rat chronic study results. Lastly, at Dr. McConnell's request, in January, 1987, I reviewed the WHO's Draft of Environmental Addendum for Environmental Health Criteria No. 29: 2,4-Dichlorophenoxyacetic Acid and provided comments to Dr. Mercier of the WHO.



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